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ORIGINAL ARTICLE

Protective Effect of the Traditional Korean Herbal Prescription, *Bojangunbi-tang*, on Non-steroidal Anti-Inflammatory Drug-induced Small Bowel Injury

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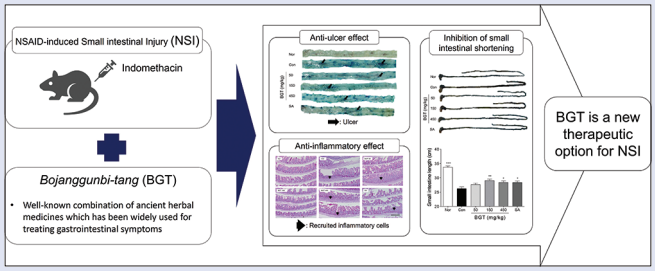
ABSTRACT

Background: *Bojanggunbi-tang* (BGT) is a well-known combination of ancient Korean herbal medicines and has been widely used for treating gastrointestinal symptoms. **Objectives:** This study was aimed at investigating whether BGT protects against non-steroidal anti-inflammatory drug (NSAID)-induced small intestinal injury (NSI), by using a murine model of indomethacin-induced NSI. **Materials and Methods:** NSI was induced in the mice by subcutaneous injections of 15 mg/kg indomethacin. BGT was administered at doses of 50, 150, and 450 mg/kg, while the positive control received sodium alginate. The treatments were orally administered twice, 30 min before and 6 h after the induction of NSI. The body weight, length of small intestine, macroscopic damages, and histological damages were assessed after 24 h of induction. **Results:** Gross anatomical analysis revealed that BGT inhibited the shortening of the small intestine and reduced the area of ulceration. Histological analysis revealed that BGT lowered the ulceration and inflammation scores. However, there was no difference between the groups with respect to weight loss. **Conclusion:** BGT ameliorated NSI via its anti-inflammatory and anti-ulcerative properties. The current study suggests that BGT could be a therapeutic option for NSI.

Key words: *Bojanggunbi-tang,* herbal medicine, inflammation, nonsteroidal anti-inflammatory drugs, small intestinal injury, ulceration

SUMMARY

- This study was aimed at investigating the protective effect of *Bojanggunbi-tang* (BGT), an herbal medicine, against nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal injury. The small intestinal injury was induced in C57B/L male mice using indomethacin. The results of our study demonstrated that BGT exhibited a protective effect against macroscopic and histological damage of inflammation and ulcer particularly at the dose of 150 mg/kg. Therefore, BGT could be an attractive option for treating diseases of the small intestine induced by NSAID.



Abbreviations used: AMK: *Atractylodes macrocephala* Koidz.; AOJ: *Alisma orientalis* Juzep.; BGT: *Bojanggunbi-tang*; COX: Cyclooxygenase; DW: Distilled water; EB: Evans blue; IBD: Inflammatory bowel diseases; IL: Interleukin; LJT: *Lonicera japonica* Thunb.; NSAID: Non-steroidal anti-inflammatory drug; NSI: Non steroidal anti-inflammatory drug-induced small intestinal injury; p.o.: Per os (orally); SA: Sodium alginate; SEM: Standard error of mean; TNF: Tumor necrosis factor.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are of undoubted benefit for the treatment of musculoskeletal disease, however, it may cause severe gastrointestinal damage including ulcer and bleeding from the stomach and small intestine.[1] Recent advancements in the diagnostic tools for small bowel pathologies, including capsule and double-balloon endoscopy, have revealed the clinical importance of NSAID-induced small intestinal injury (NSI).[2] It has been reported that approximately 70% of chronic NSAID users have significant small intestinal damage, and the damage is sub-clinical in most of them.[3,4] However, due to the complexity of the pathogenesis of NSI, current therapeutic treatments have been shown inefficient effects including acid suppressants, selective cyclooxygenase (COX)-II inhibitors, and prostaglandin-analogs. Prostaglandin induces diarrhea and is contraindicated in women of childbearing,[5] it has been proven that the ability of COX-II inhibitors to damage the small bowel is comparable to that of no-selective NSIADs,[6] and even the exacerbation of small intestinal damage with proton-pump inhibitors was observed in recent studies.[7,8] Therefore, various preventive and protective strategies, including traditional medicine, have been considered for the treatment of NSI.[9,10]

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Bojanggunbi-tang (BGT) is a traditional Korean herbal medicine consisting of *Lonicera japonica, Atractylodes macrocephala, Paeonia lactiflora, D. lablab, Dioscorea japonica, Crataegus pinnatifida, Poria cocos, Magnolia officinalis, Citrus unshiu, Alisma orientalis, Massa medicata, Hordeum vulgare, Zingiber officinale, Aucklandia lappa, Amomum villosum* and *Glycyrrhiza uralensis*. It has been clinically used for the treatment of gastrointestinal symptoms, including abdominal pain, indigestion, and diarrhea in Korea.[11] We have previously reported the ameliorative effect of BGT on murine models of colitis,[12] and the alleviative effect of *L. japonica*, the main ingredient of BGT, on a murine model of dextran sulfate sodium-induced colitis.[13] We hypothesized that BGT would be also effective in treating NSI, as it is accompanied by ulceration and inflammation. We investigated this notion by performing an experimental study using a murine model of indomethacin-induced small intestinal injury.

MATERIALS AND METHODS

Animals

C57B/L male mice (6 w, 20 ± 2 g) were purchased from Daehan Bio Link (Seoul, Korea). The mice were housed at an ambient temperature of 20°C–22°C and 50% ± 10% relative humidity and had *ad libitum* access to food (Samyang, Korea) and water. The mice were acclimated for 5 d before experimentation. All the experimental procedures were performed in accordance with the International Animal Ethical Committee of Kyung Hee University, and the experimental protocol was approved by the Committee (approval number: KHUASP (SE)-19-273).

Sample preparation and grouping

Each crude herbal medicine composed of BGT was prepared from Kyung Hee Hanyak Co. (Seoul, Korea). Each daily dose of BGT applied in humans is comprised of flowers of *L. japonica* Thunb. (LJT, 40 g), roots of *A. macrocephala* Koidz. (AMK, 16 g), roots of *P. lactiflora* Pall. (16 g), seeds of *Dolichos lablab* L. (16 g), roots of *D. japonica* Thunb. (16 g), fruits of *C. pinnatifida* Bunge var. typica Schneider (16 g), *P. cocos* Wolf (16 g), stem bark of *M. officinalis* Rehder and Wilson (12 g), cortex of *C. unshiu* Marcovich (12 g), roots of *A. orientalis* Juzep. (AOJ, 12 g), *M. medicata* Fermentata (8 g), seeds of *H. vulgare* Linne (8 g), roots of *Z. officinale* Roscoe (8 g), roots of *A. lappa* Decne.(6 g), fruits of *A. villosum* Lour (6 g), and root of *G. uralensis* Fisch.(4 g) as detailed in the previous study.[12] Among these, LJT, AMK, and AOJ were quantitively analyzed the major compounds previously using high-pressure liquid chromatography and revealed that LJT, AMK, and AOJ included 42.2 μg/mg chlorogenic acid, 75.1 ng/mg atractylenolide III, and 624 ng/mg alisol B acetate, respectively (article in press).

Total 216 g of the herbal mixture was extracted by boiling in distilled water (DW) at 100°C for 2 h, subsequently filtered, and freeze-dried to a powdered form. The sample was stored in the laboratory of herbal pharmacology in Kyung Hee University. The mice were divided into 6 groups, namely, the normal, control (DW, p.o.), sodium alginate-treated (SA, positive control, 200 mg/kg, p.o.), and 3 BGT-treated (50, 150, or 450 mg/kg, p.o.) groups (n = 10, each).

Induction of nonsteroidal anti-inflammatory drug-induced small intestinal injury

NSI was induced by using a previously described method, with minor modifications.[14] Indomethacin (Sigma, USA) was dissolved in 0.01 M Na_2CO_3 and subcutaneously injected at a dose of 15 mg/kg (150 μl volume) into mice that had been made to fast for 18 h. The samples were dissolved in DW and administered twice: 30 min before and 6 h after the induction of NSI. The control group was administered the same volume of DW by the same method.

Assessment of weight and gross anatomical analysis

The body weight was measured thrice (before induction, at 0 d, and 1 d after the induction of NSI). Evans blue (EB, 1%, 50 μl, i.v.) was injected 30 min before sacrificing the mice. The small intestine was subsequently isolated and the length was measured after 24 h of induction. The number of EB dots and the area of the dots (cm^2) were measured by the Image J software (National Institutes of Health, Bethesda, MD).

Histological analyses

The small intestine was dissected into 2 pieces, and the distal half was postfixed in 4% paraformaldehyde for 1 d and rolled using the swiss roll method.[15] For each roll, the paraffin block was sliced into 4 μm-thick sections and stained with periodic acid-Schiff using a previously described method.[14] Three investigators, who were blind to the protocol, scored the histological lesions according to a modified scoring system reported in a previous study.[16] The severity of inflammation or ulceration was graded on a scale of 0–4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe). The total score was calculated by summing the inflammation and ulceration scores.

Statistical analyses

All the results were expressed as the mean ± standard error of mean. The data were statistically analyzed by one-way analysis of variance followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS

Assessment of weight and gross anatomical analysis

With the exception of the normal group, the changes in body weight were not significantly different between the groups [Figure 1a]. The length of the small intestine in the control group was reduced by 22.1% compared to that of the normal group. The shortening of the small intestine was significantly inhibited by 150 and 450 mg/kg BGT and SA, compared to that of the control group [$P < 0.05$ and $P < 0.01$, Figure 1b and c].

Gross anatomical analysis revealed the presence of ulcers in all the groups, except for the normal group [Figure 1d]. The reduction in the area of ulceration of the groups treated with 50, 150, and 450 mg/kg BGT and SA group was statistically significant [$P < 0.05$ and $P < 0.01$, Figure 1e]. However, there were no differences between the groups with respect to the number of ulcerative patches [Figure 1f].

Histological analyses

The indomethacin-treated mice showed epithelial damage, alterations in the villi, infiltration of inflammatory cells, mucosal and submucosal disruption, and thickening of the intestinal wall unlike normal group [Figure 2a]. These pathological changes were markedly improved by treatment with BGT [Figure 2a]. The inflammation score, ulceration score, and total score were reduced in the groups treated with BGT and SA [$P < 0.05$ and $P < 0.01$, Figure 2b-d]. The maximum effects were observed in the group treated with 150 mg/kg BGT and were similar to those of the SA-treated group [Figure 2b and d].

DISCUSSION

In the present study, we have investigated the effect of BGT on NSI. We found that BGT inhibited the shortening of small intestinal length. The administration of indomethacin led to macroscopic ulcers, and

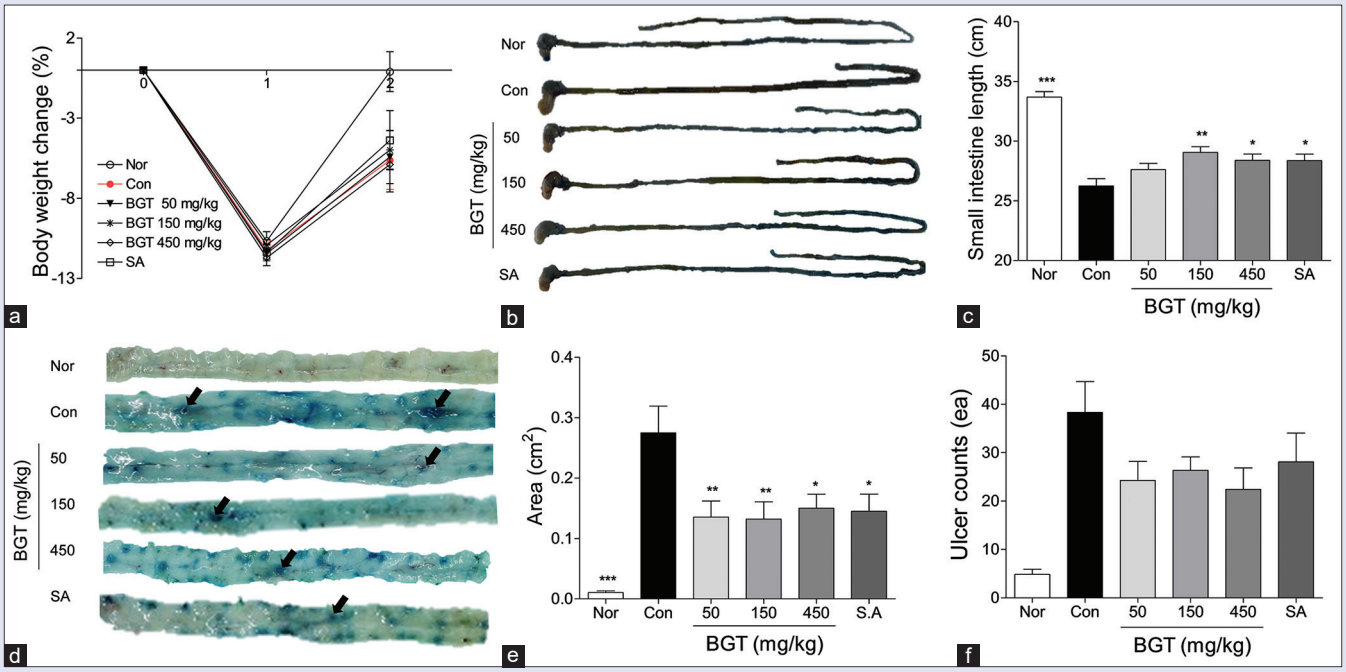

Figure 1: Changes in the length of small intestine, body weight, and macroscopic damages in mice model of indomethacin-induced small intestinal injury. (a) Indicates the body weight change. (b) Indicates the representative photo of small intestine and (c) indicates the graph of the length of small intestine. (d) indicates the representative photo of macroscopic damage and (e and f) indicate the graph of ulcer area and number. Arrows indicate the ulceration stained with Evans blue (d). Data are expressed as mean ± standard error of mean. *P < 0.05 and **P < 0.01 compared with the control group (n = 10)

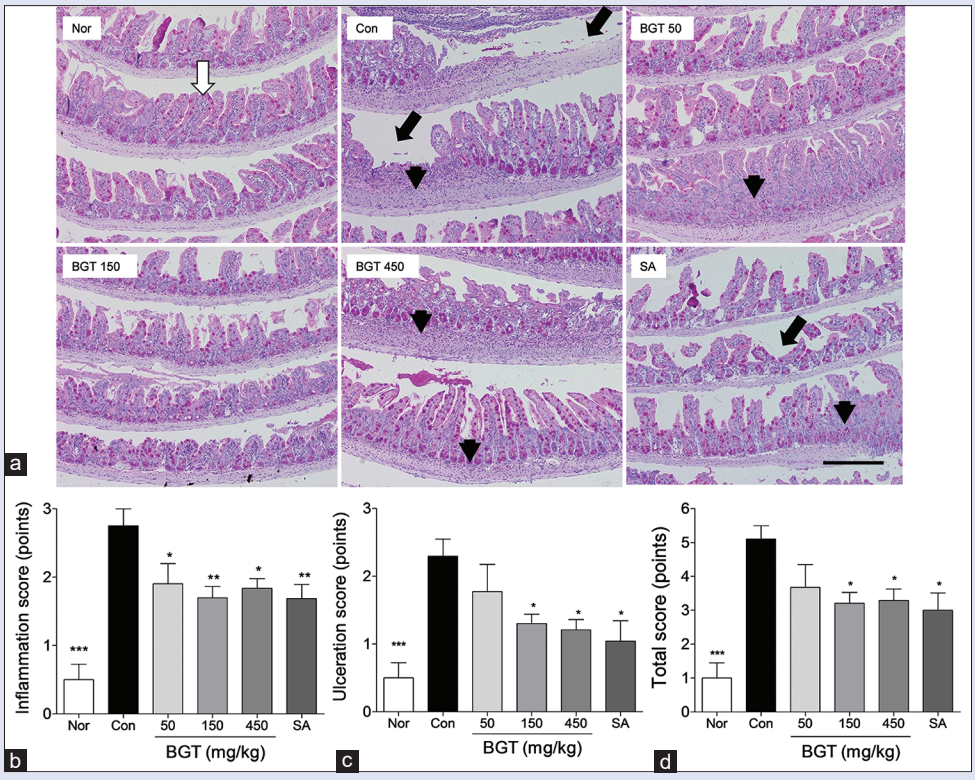

Figure 2: Changes in scores of histological damages in mice model of indomethacin-induced small intestinal injury. (a) Indicates the representative histological photo of small intestine of each group (×100). (b-d) Indicate the graphs of inflammation, ulceration and total scores. White arrows indicate the intact villi, black arrows indicate the ulceration, and arrowheads indicate the recruited inflammatory cells in damaged area. Black scale bar in (a) is 200 μm. Data are expressed as mean ± standard error of mean. *P < 0.05 and **P < 0.01 compared with the control group (n = 10)

histological inflammatory cell infiltration, and disruption in mucosa and submucosa. BGT exhibited a protective effect against macroscopic and histological damage of inflammation and ulcer particularly at the dose of 150 mg/kg.

The pathology of NSI is complex and has not been fully elucidated.[17] The several important mechanisms have been reported in NSI; the reduction of intestinal mucus due to prostaglandin E_2 depletion, increased intestinal motility, nitric oxide, neutrophil infiltration, inflammatory cytokines, and reactive oxygen species.[17,18] Studies in animal models have suggested a variety of possible approaches such as anti/probiotics and complementary medicines to protect and prevent NSAID-enteropathy.[14,19,20] Based on previous studies,[14,20] we carried out pilot experiments using 2 positive controls; rifaximin and sodium alginate. Rifaximin, characterized by very low gastrointestine-absorbed antibiotics, can correct the shift of intestinal microflora toward pro-inflammatory Gram-negative bacteria leading to preventing NSAID-enteropathy.[20] Sodium alginate, a soluble dietary fiber extracted form brown seaweed, protected against depletion of mucosal mucins induced by NSAID.[14] Sodium alginate showed a more protective effect to indomethacin compared to rifaximin in our preliminary experiment, thus we chose a sodium alginate as a positive control. We also attempted to find proper time points of sacrifice after administration of indomethacin due to that several time points have been evaluated in previous studies.[21,22] We chose 24-h time point after indomethacin administration because the shorted small bowel length was the most evident at this time, and there was a tendency of recovery in small intestinal length after a day. We tried various doses in a subsequent preliminary study, including 7.5, 10, and 15 mg/kg and we have failed to find shortening of small intestine and mucosal damage except for at 15 mg/kg dose at pilot experiment. Finally, we adopted 15 mg/kg of indomethacin for this experiment. The histological damage was only investigated in the back half of the small intestine, because the majority of bleeding and damage induced by NSAIDs occurs in the distal small intestine,[23,24] where NSIAD is re-absorbed related to de-conjugation of NSAID, which allows the NSAID to be transported across the epithelium.[25] In clinical settings, the damage is mostly transferred to the distal small intestine due to the wide use of enteric-coated aspirin.[26]

BGT is a combined prescription of ancient famous herbal medicine '*Daehwajungeum*' and "*Sambaek-tang*" and has been widely used for the treatment of acute gastritis, colitis, and irritable bowel syndrome.[11] Researchers put eye on its extensive pharmacological effects on inflammatory bowel diseases (IBD), and *L. japonica*, a major active constituent of BGT, has been reported to take part in down-regulation of interleukin-1 beta (IL-1 β), Tumor necrosis factor-alpha (TNF-α), interferon-γ, IL-6, IL-12 and IL-17 leading to prophylactic effect against dextran sulfate sodium-induced colitis.[13] The proinflammatory cytokines such as IL-1 β, TNF-α play a key role in the pathogenesis of NSI in clinically,[27] and experimentally.[28] Considering that similarity between NSI and IBD is represented macro- and microscopically, and they also sensitive the same medicines (e.g., corticosteroids, sulphasalazine),[29] there is a possibility that BGT has ameliorative effect against NSI through a similar mechanism for suppressing pro-inflammatory cytokines of IBD. A recent studies showed that widely prescribed traditional herbal medicine with BGT to cure IBD, *Hwangryunhaedoktang* (*Orengedokuto* in kampo medicine and *Huan-Lian-Jie-Du-Tang* in Chinese Medicine), can protect the intestinal mucosa of NSAID users through repairing of the enteric nervous system, relaxing nitric oxide, and upregulation the production of prostaglandin E_2.[9,10] The broad exploratory research on the mechanism of BGT to NSI is needed.

In summary, administration of BGT with the indomethacin prevents NSI. Although the further mechanisms of BGT are needed to be investigated, our experimental study supports the BGT can be an attractive avenue to protect small bowel against NSAID-induced injury.

CONCLUSION

The results of our study demonstrated that BGT could ameliorate NSI via its anti-inflammatory and anti-ulcerative properties. The mechanisms underlying the ameliorative effects of BGT need to be further investigated. The results of our present and previous studies demonstrate that BGT could be an attractive option for treating diseases of the small and large intestines.

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Conflicts of interest

There are no conflicts of interest.

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